REMARKS

Applicants through their attorneys appreciate the opportunity to discuss this case with the Examiner on August 13, 2002.

Applicants have cancelled Claims 5, 6, 7, 15, 16, 32, 36, 37, 38, 46, and 47. Applicants reserve the right to prosecute canceled claims covering subject matter, not covered in the present application, in a continuation application.

Applicants have amended the claims to clearly cover preferred embodiments of Applicants' invention for reducing loss of affinity particles used in one or more steps of a method for isolating proteinaceous molecules. In particular, the claims of this application are directed to a method for isolating a fusion protein comprising a peptide, polypeptide, or protein and a metal chelating group consisting of a plurality of consecutive histidine residues using metal-chelate affinity particles in the presence of a detergent in the range of 0.0005 - 2 (v/v) % sufficient to reduce loss of the metal-chelate affinity particles during a separation step (see, Claims 2, 34, 64, and 66, and claims depending therefrom). In another preferred embodiment, the metal-chelate affinity particles are magnetic (see, Claims 64 and 66, and claims depending therefrom). Furthermore, in a particularly preferred embodiment, the invention provides a method for reducing loss of metal-chelate affinity particles wherein the metal-chelate affinity particles are nickel-nitrilotriacetic acid (Ni-NTA) agarose beads (see, new Claim 70). Support for the amendments to the claims to cover the various preferred embodiments of this invention for reducing metal-chelate affinity particle loss using a detergent in a method for isolating a fusion protein is found in the specification (see, e.g., p. 8, lines 20-22; p. 9, lines 1-30; p. 11, lines 1-14; Examples 1-6 at p. 20, line 1-p. 28, line 24 of the specification). Accordingly, the amendments add no new matter.

Applicants have amended Claims 8, 39, and 48, to adjust dependencies from canceled claims to an appropriate claim carried forward in this application. Accordingly, the amendments add no new matter.

Applicants have also amended Claims 13, 14, 19, 44, and 45 to incorporate terms used in the claims from which they depend. Accordingly, the amendments are made to maintain consistent use of terms throughout the claims and, thus, add no new matter.

Entry of the amendments is respectfully requested.

In the Office Action, the Examiner maintained rejections of certain claims under 35 U.S.C. § 102 as anticipated by Zhang or Weisburg. The Examiner also maintained his rejections of certain claims under 35 U.S.C. § 103 as obvious over Weisburg and also over the combinations

of Zhang and McCoy, Zhang and Gallant, Zhang and Stein, Zhang and Tsaur, and Zhang and Taoda.

Applicants have previously reviewed and characterized the references and combinations of references relied on the by the Examiner (see, Applicants' prior Response, dated November 19, 2001). Applicants have explained that none the references either alone or in the combinations cited in the Office Action teach or suggest using detergent to reduce affinity particle loss in a method for isolating a peptide, polypeptide, or protein. Furthermore, in the case of rejections under 35 U.S.C. § 103, Applicants noted the complete lack of any evidence as to why persons skilled in this art would even be motivated to combine the references as envisioned by the Examiner to arrive at Applicants' invention. Applicants now respectfully traverse the current rejections for the reasons already of record and for the additional reasons indicated below.

At the outset, Applicants note that their invention is a ***method*** that employs a particular range of detergent to reduce loss of metal-chelate affinity particles in a method for isolating fusion proteins. The inventive feature of Applicants' claimed invention does ***not*** reside in one or a combination of elements of a ***composition***. The compositions employed in the claimed invention are well known to persons skilled in this art. Applicants have discovered ***how*** to reduce losing metal-chelate affinity particles; a phenomenon that can occur when such particles are employed to isolate fusion proteins designed to bind to the metal-chelate affinity particles. Accordingly, the claimed method of Applicants' invention effectively increases yields of fusion proteins isolated by metal-chelate affinity procedures.

As noted above, the claims of this application are now directed to preferred embodiments of Applicants' method, i.e., wherein the detergent is used in the range of 0.0005 - 2 (v/v) % to reduce loss of metal-chelate affinity particles in a method for isolating a fusion protein comprising a peptide, polypeptide, or protein and wherein a metal chelating group of the fusion protein consists of a plurality histidine residues. Particularly preferred embodiments as claimed herein include methods comprising the use of metal-chelate, magnetic affinity particles, the use of a fusion protein comprising a metal chelating group consisting of six consecutive histidine residues, and the use of a particular species of metal-chelate affinity particles, i.e., nickel-nitriloacetic acid (NTA) agarose beads.

Nowhere does Zhang's method of detecting targeted nucleic acids provide a teaching or suggestion of the preferred embodiments of Applicants' invention for reducing affinity particle loss as claimed herein. Similarly, Weisburg's description of a standard lysis buffer containing a detergent in a method of identifying ribosomal RNA and genes in spirochetes fails to teach each

and every element of Applicants' claimed methods. Accordingly, both references fail to teach and suggest the claimed invention.

McCoy's description of using metal-chelate affinity beads fails to even mention that affinity particles can be lost during any step in an isolation procedure. Since McCoy cannot solve what it does not even recognize as a problem, it does not cure the deficiency in Zhang to provide Applicants' claimed invention to those of ordinary skill in this art.

Gallant's description of using the detergent CHAPS in an HPLC protocol for purifying an enzyme or the production of new inhibitors of the enzyme apopain also completely fail to recognize the problem of how to reduce loss of metal-chelate affinity particles in a method for isolating fusion proteins. Accordingly, Gallant in combination with the nucleic acid isolation methods of Zhang does not suggest Applicants' claimed methods.

Stein's description of using cationic wetting agents in a continuous method of separating complex mixtures of fatty alcohols based on differential melting temperatures provides no relevant teaching or suggestion for how a person skilled in this art might modify the methods of isolating target nucleic acids according to Zhang to arrive at Applicants' claimed methods that reduce loss of metal-chelate affinity particles in methods for isolating fusion proteins.

Similarly, Tsaur's description of a polyvinyl alcohol or various hydrophilic polymers that can reversibly cross-link with the hydrophobic polymer core of a composite detergent composition and Taoda's description of an environment purifying material in which particles of titanium oxide (photocatalyst) coated with calcium phosphate to decompose proteins, bacteria, and viruses, all fail to provide any relevant suggestion that would transform the teaching of Zhang into Applicants' claimed methods.

The above comments clearly show that none of the references or combinations thereof cited by the Examiner suggests Applicants' claimed ***methods***. The Examiner urges that the steps of Applicants' ***method*** are suggested by a listing of ***compositions***, without disclosure of Applicants' problem or solution. This is reading too much life into inert references. Accordingly, reconsideration and withdrawal of the rejections is respectfully requested.

The issue in this case is whether Applicants' claimed invention for reducing metal-chelate affinity particle loss in methods for isolating fusion proteins can be said to be inherently described in the examples of the references. Applicants' view is that none of the references describes the problem of losing affinity particles or Applicants' method of solving this technical problem. It cannot be determined whether any reference or combination of references experienced the phenomenon of losing affinity particles as a technical problem recognized by Applicants. The Examiner apparently argues that the references inherently describe Applicants'

methods merely because affinity particles or various detergents recited in Applicants' method claims are listed. However, as Applicants have noted previously, the claimed invention is not a composition of listed materials new to the art. None of the materials or combination thereof have ever been asserted to be new. Rather, Applicants have claimed a particular ***method*** to overcome a particular problem that is not even recognized in any of the references relied on by the Examiner.

In view of all of the above comments, Applicants respectfully submit that the claims are in proper form for allowance. Accordingly, the Examiner is respectfully requested to enter the amendments, withdraw the rejections, and pass the claims to allowance.

Respectfully submitted,

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CERTIFICATE OF MAILING

The undersigned hereby certifies that the items of correspondence referred to above are being deposited with the U.S. Postal Service First Class Mail, postage prepaid, in an envelope addressed to the Commissioner for Patents, Box AF, Washington, DC 20231 on the date indicated below:

September 3, 2002 — Thomas R. Berka

date of mailing and signature — Thomas R. Berka

AMENDED CLAIMS IN U.S. Serial No. 09/812,541

(marked up claims showing deletions and cancellations by ~~strikethrough~~, additions by underlining, and new claims as indicated)

2. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein and a metal chelating group consisting of a plurality of consecutive histidine residues ~~molecule~~ from a sample in a vessel, comprising the steps of:

(a) combining the sample containing ~~a~~ the fusion protein ~~peptide, polypeptide, or protein molecule of interest~~ with metal-chelate affinity particles suitable for binding said fusion protein ~~molecule~~, said affinity particles being insoluble in the sample;

(b) collecting the metal-chelate affinity particles;

(c) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(d) optionally, resuspending the metal-chelate affinity particles in a solution;

(e) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein ~~molecule~~;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005 - 2 (v/v) % detergent sufficient to reduce loss of metal-chelate affinity particles during any separation step, in comparison to the same method performed in the absence of detergent.

~~5. The method according to Claim 2, wherein said molecule is a fusion protein or peptide.~~

~~6. The method according to Claim 5, wherein said fusion protein is a protein or peptide fused to a metal chelating group.~~

~~7. The method according to Claim 6, wherein said metal chelating group is two or more histidine residues.~~

8. (amended) The method according to Claim 2 ~~6~~, wherein said metal chelating group is six consecutive histidine residues.

13. (amended) The method according to Claim 2, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

14. (twice amended) The method according to Claim 2, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.

~~15. The method according to Claim 14, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides, nucleotides or small molecules capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.~~

~~16. The method according to Claim 2, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).~~

17. (amended) The method according to Claim 2 ~~16~~, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

19. (twice amended) The method according to Claim 17, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

~~32. The method according to Claim 2, wherein the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).~~

34. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and a metal chelating group consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate affinity particles and incubating said metal-chelate affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with metal-chelate affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate affinity particles being insoluble in the sample;

(c) collecting the metal-chelate affinity particles;

(d) separating the metal-chelate affinity particles from the unbound remainder of the sample;

(e) optionally, resuspending the metal-chelate affinity particles in a solution;

(f) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate affinity particles, followed by separating the metal-chelate affinity particles from said eluted fusion protein ~~molecule~~;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005 - 2 (v/v) % detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate affinity particles during any separation step, in comparison to the same method performed in the absence of detergent.

~~36. The method according to Claim 34, wherein said molecule is a fusion protein or peptide.~~

~~37. The method according to Claim 36, wherein said fusion protein is a protein or peptide fused to a metal chelating group.~~

~~38. The method according to Claim 37, wherein said metal chelating group is two or more histidine residues.~~

39. (amended) The method according to Claim 34 ~~37~~, wherein said metal chelating group is six consecutive histidine residues.

44. (twice amended) The method according to Claim 34, wherein said metal-chelate affinity particles are selected from the group consisting of ferromagnetic beads,

superparamagnetic beads, and combinations thereof.

45. (twice amended) The method according to Claim 34, wherein said metal-chelate affinity particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, combinations thereof.

~~46. The method according to Claim 45, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.~~

~~47. The method according to Claim 34, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).~~

48. (amended) The method according to Claim 34 ~~47~~, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.

64. (twice amended) A method for isolating a fusion protein, wherein said fusion protein comprises a peptide, polypeptide, or protein molecule and a metal chelating group consisting of a plurality of consecutive histidine residues from a sample in a vessel, comprising the steps of:

(a) combining the sample containing ~~a~~ the fusion protein ~~peptide, polypeptide, or protein molecule of interest~~ with metal-chelate, magnetic affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(b) applying a magnetic field to the vessel so as to attract and immobilize the metal-chelate, magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(d) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(e) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate, magnetic affinity particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein ~~molecule~~;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of 0.0005 - 2 (v/v) % detergent sufficient to reduce loss of metal-chelate, magnetic affinity particles during any separation step, in comparison to the same method performed in the absence of detergent.

66. (twice amended) A method for isolating a fusion protein comprising a peptide, polypeptide, or protein and a metal chelating group consisting of a plurality of consecutive histidine residues ~~molecule~~ from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of metal-chelate, magnetic affinity particles and incubating said metal-chelate, magnetic affinity particles in the presence of a detergent;

(b) combining the sample containing the fusion protein ~~a peptide, polypeptide, or protein molecule of interest~~ with said metal-chelate, magnetic affinity particles suitable for binding said fusion protein ~~molecule~~, said metal-chelate, magnetic affinity particles being insoluble in the sample;

(c) immobilizing the metal-chelate, magnetic affinity particles by applying a magnet to said vessel;

(d) separating the remainder of the sample from the immobilized metal-chelate, magnetic affinity particles;

(e) optionally, resuspending the metal-chelate, magnetic affinity particles in a solution;

(f) optionally, eluting said fusion protein ~~molecule~~ from the metal-chelate, magnetic affinity particles, followed by separating the metal-chelate, magnetic affinity particles from said eluted fusion protein ~~molecule~~;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of 0.0005 - 2 (v/v) % detergent, wherein the use of detergent is sufficient to reduce loss of metal-chelate, magnetic, affinity particles during any separation step, in comparison to the same method performed in the absence of detergent.

70. (new) The method for isolating a fusion protein according to any one of Claims 64-66, wherein said fusion protein comprises a peptide, polypeptide, or protein and a metal chelating group consisting of six consecutive histidine residues and said metal-chelate, magnetic affinity particles are nickel-nitrilotriacetic acid agarose beads.